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## **A novel single-cell tool for absolute in situ quantification of intracellular poly-P and other storage polymers in wastewater systems key organisms**

Warnakulasuriya, Eustace Yrosh Fernando; McIlroy, Simon Jon; Nierychlo, Marta; Herbst, Florian-Alexander; Petriglieri, Francesca; Schmid, Markus; Wagner, Michael; Nielsen, Jeppe Lund; Nielsen, Per Halkjær

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# A novel single-cell tool for absolute *in situ* quantification of intracellular poly-P and other storage polymers in wastewater systems key organisms

E. Fernando <sup>1</sup>, M. Nierychlo <sup>1</sup>, S.J. McIlroy <sup>1</sup>, F-A. Herbst <sup>1</sup>, Francesca Petriglieri <sup>1</sup>, M. Schmid <sup>2</sup>, M. Wagner <sup>2</sup>, J.L. Nielsen <sup>1</sup> and P.H. Nielsen <sup>1</sup>  
<sup>1</sup> Center for Microbial Communities, Aalborg University, Denmark , <sup>2</sup> Department of Microbial Ecology and Ecosystem Science, University of Vienna, Austria



## Background and Aim

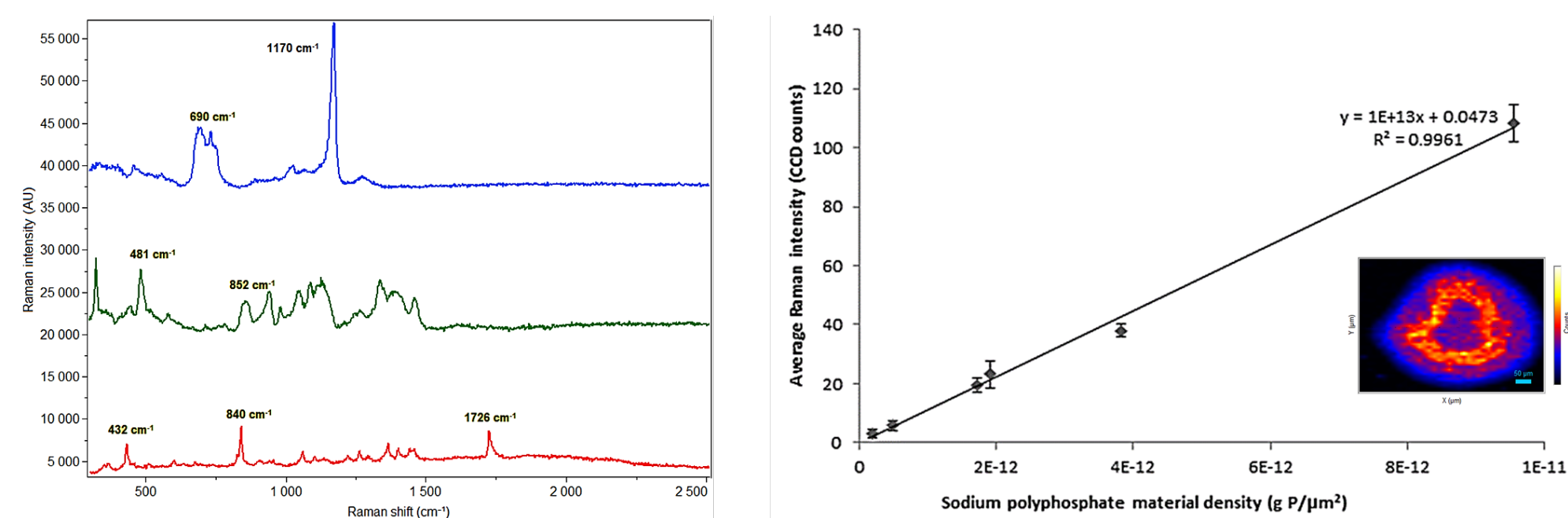
Enhanced Biological Phosphorus Removal (EBPR) is a process widely applied in wastewater treatment and relies on the ability of some microorganisms to store phosphate intracellularly. Among them, *Candidatus* Accumulibacter and *Tetrasphaera* are found worldwide, but more insights are needed on their individual contribution to the EBPR process. **This study aims** to develop a novel approach, which combines Fluorescence *in situ* Hybridization (FISH) and Raman microspectroscopy, to provide *in situ* and absolute quantification of intracellular storage compounds relevant for the EBPR process.

## Conclusions

- *Ca. Accumulibacter* is capable to store up to 3 times more than *Tetrasphaera*. However, *Tetrasphaera* cells are more abundant in some Danish EBPR plants and therefore, both genera appear to be equally important for the EBPR process.
- This novel approach provides a powerful tool for microbial ecologists and can be applied to quantify storage compounds in other microbial systems.

## Methods

Raman microspectroscopy and calibration of the instrument

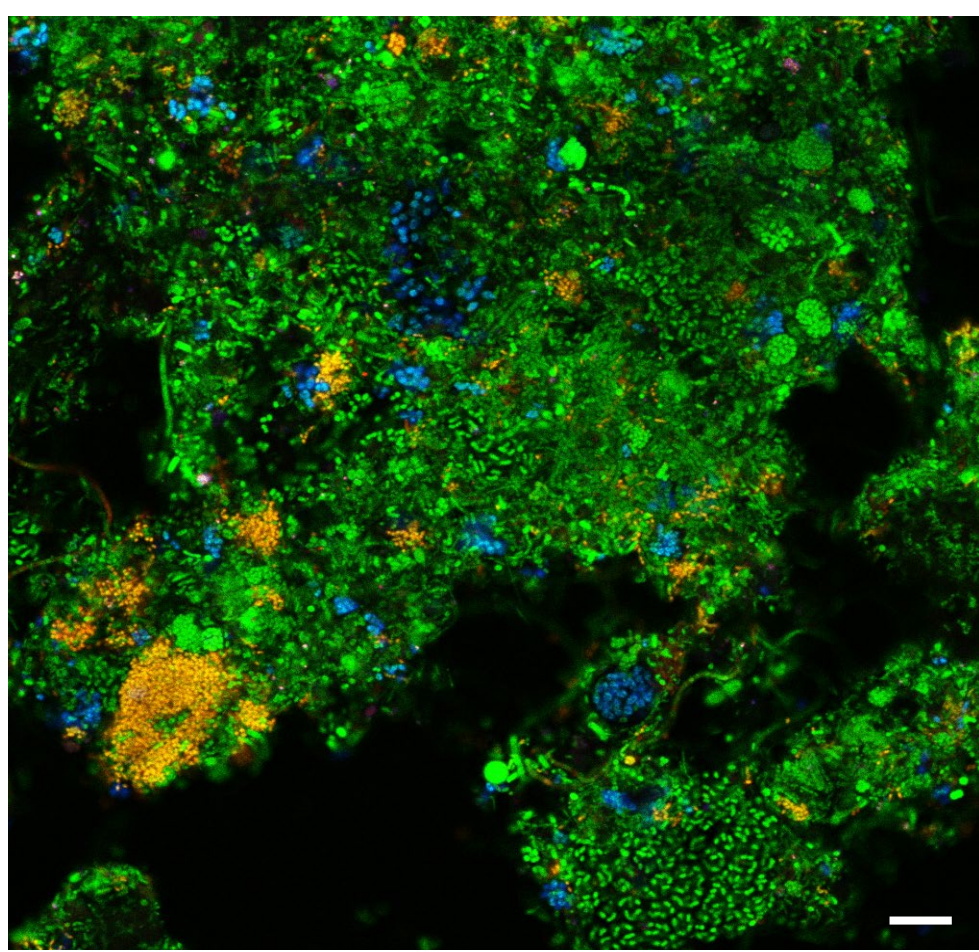


Validation of the quantification method →  
P-uptake/release experiment with *Tetrasphaera elongata* and activated sludge

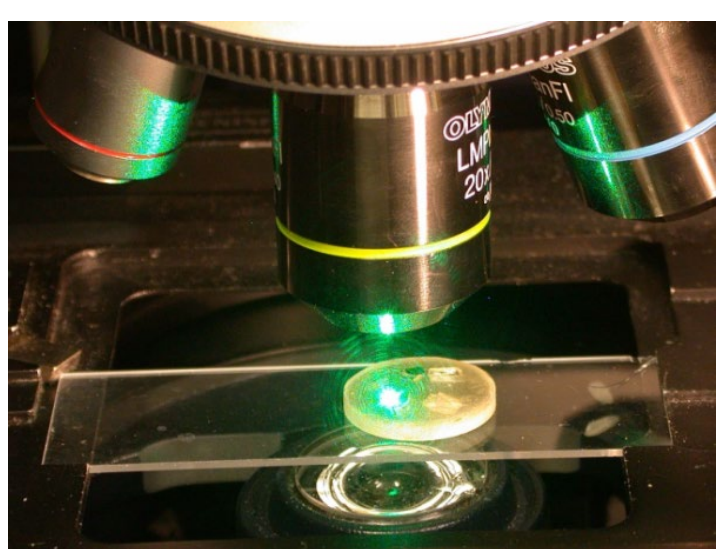
Quantification *in situ* using FISH-Raman



Samples were obtained from 8 different EBPR plants at different process stages.



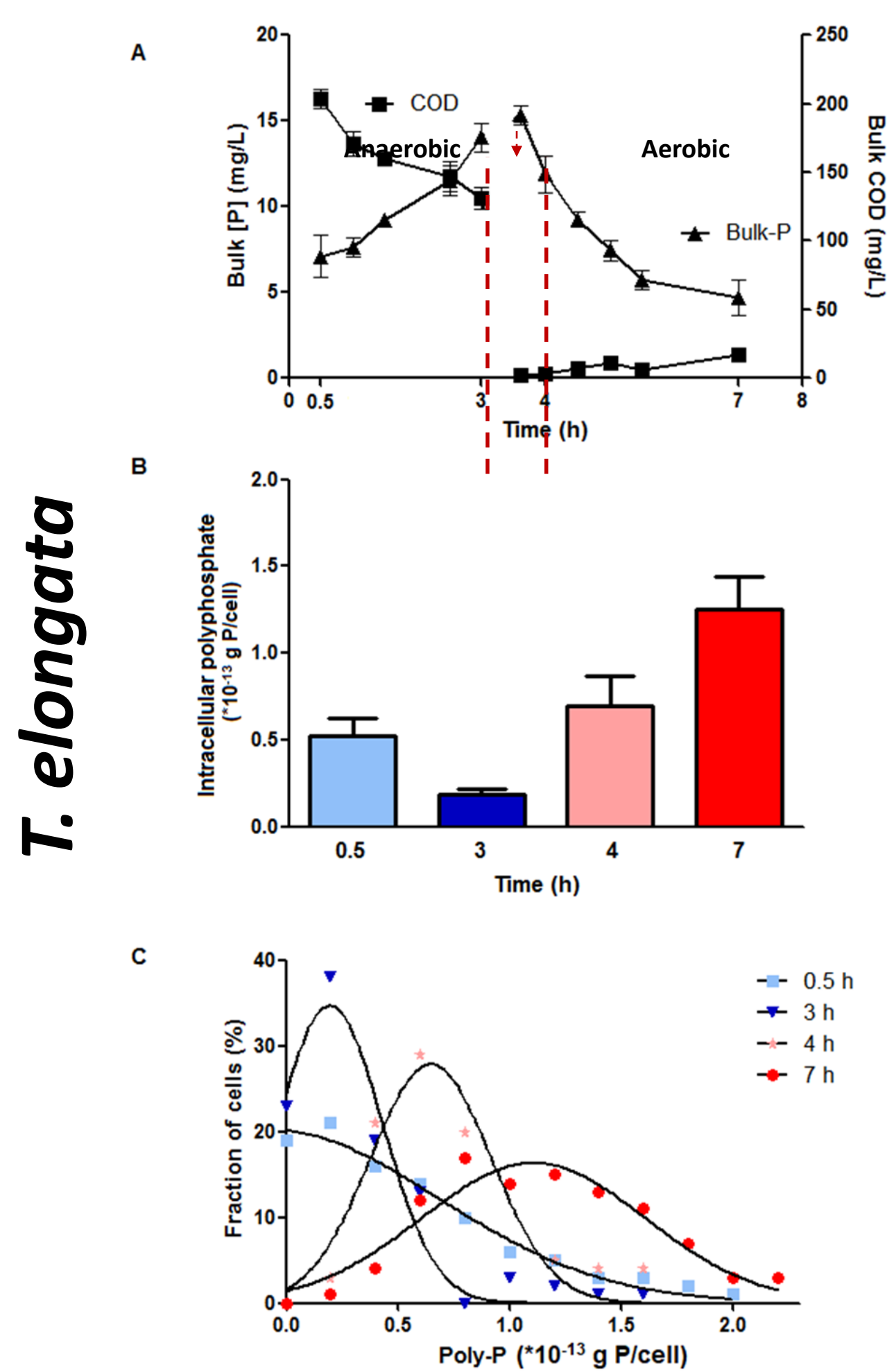
FISH was performed using the probes PAO651 and Actino658 for *Ca. Accumulibacter* and *Tetrasphaera*, respectively.



FISH-positive cells were bleached and Raman spectra were recorded from the target cells.

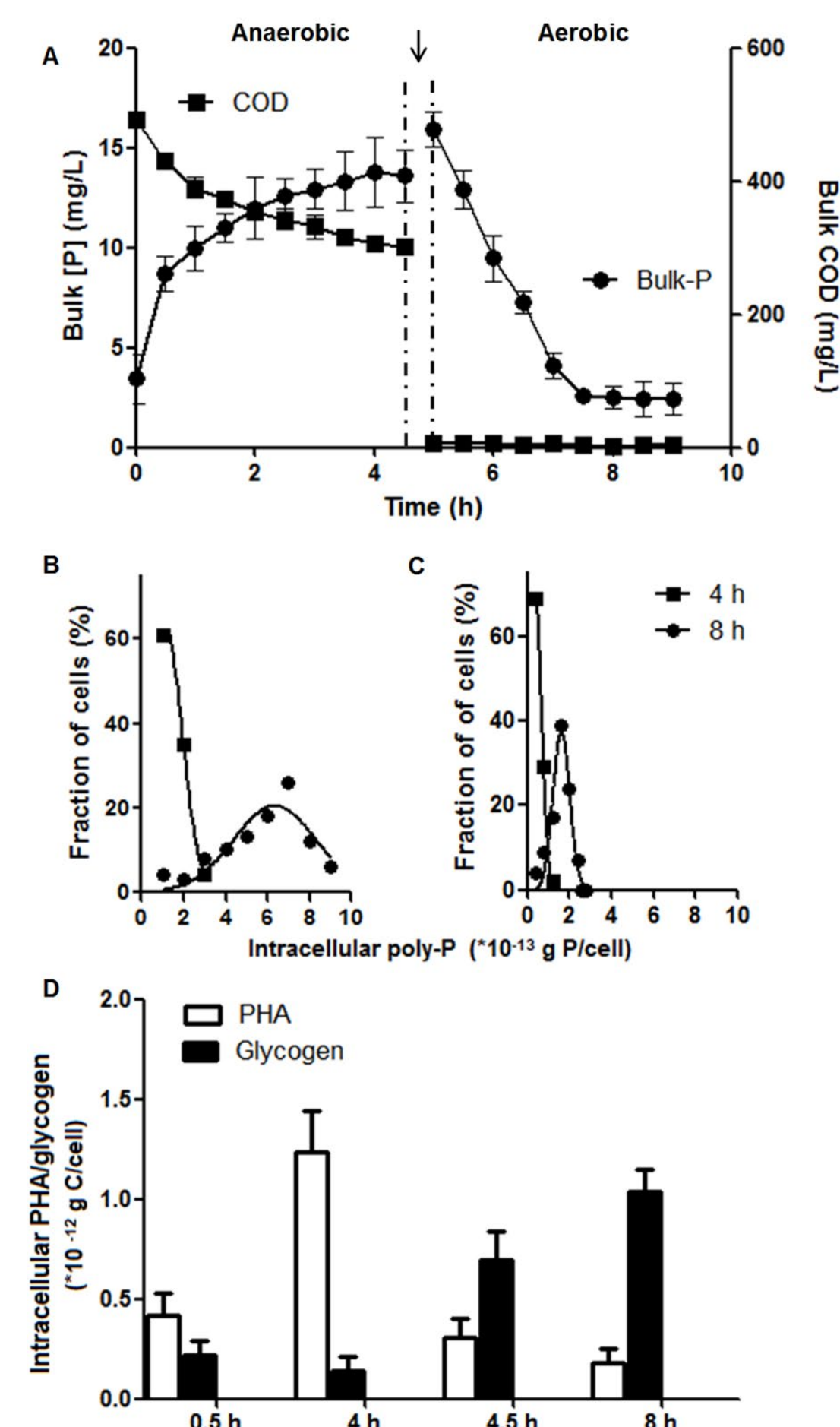
$$[\text{Poly-P}] = k * \sum S * A_{\text{cell}}$$

## Lab-scale P-uptake/release experiment confirms dynamic intracellular-P behaviour



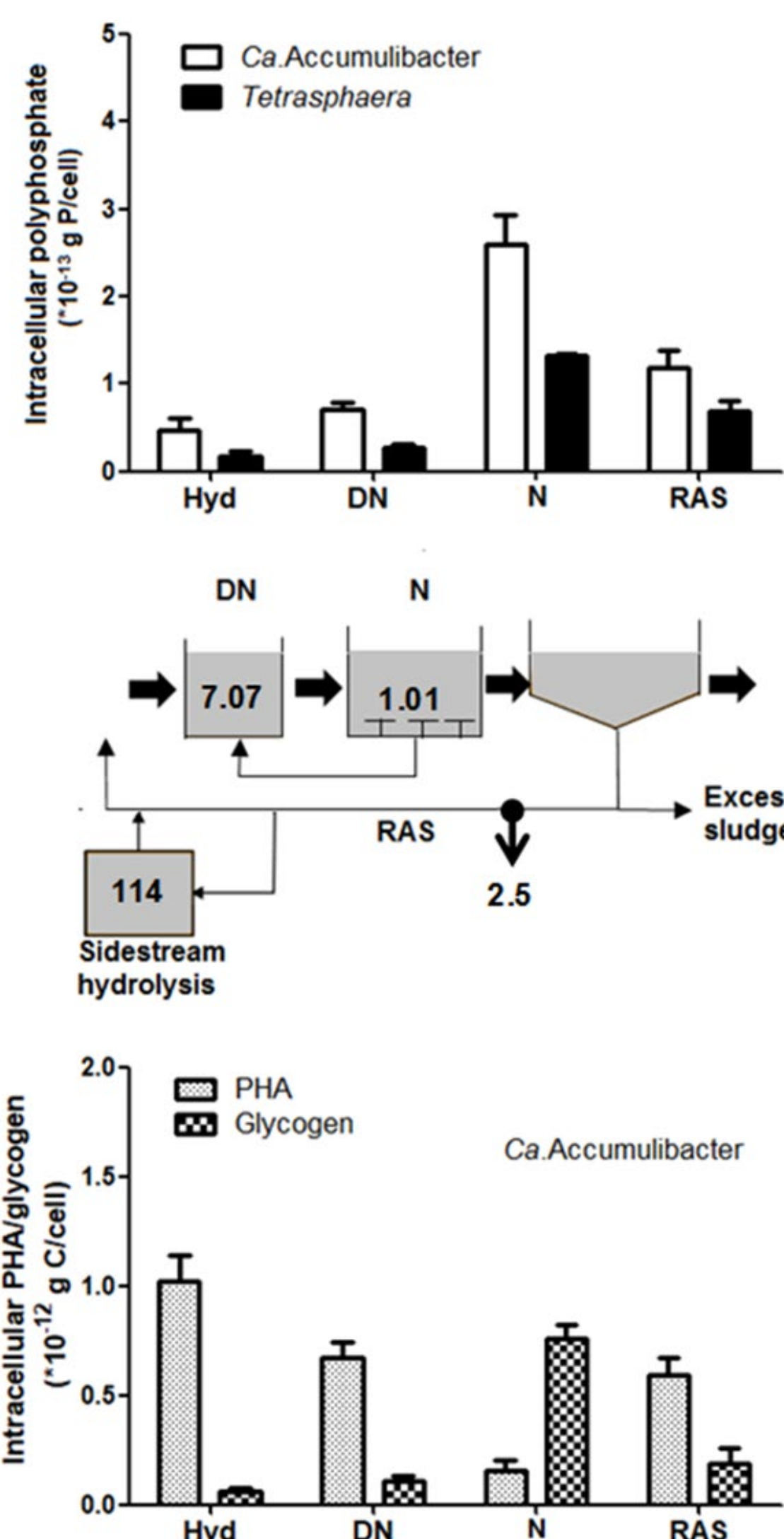
The fluctuations of ortho-P concentration in the bulk medium (A) reflected the Raman-based quantifications of intracellular poly-P content (B-C).

## Activated sludge

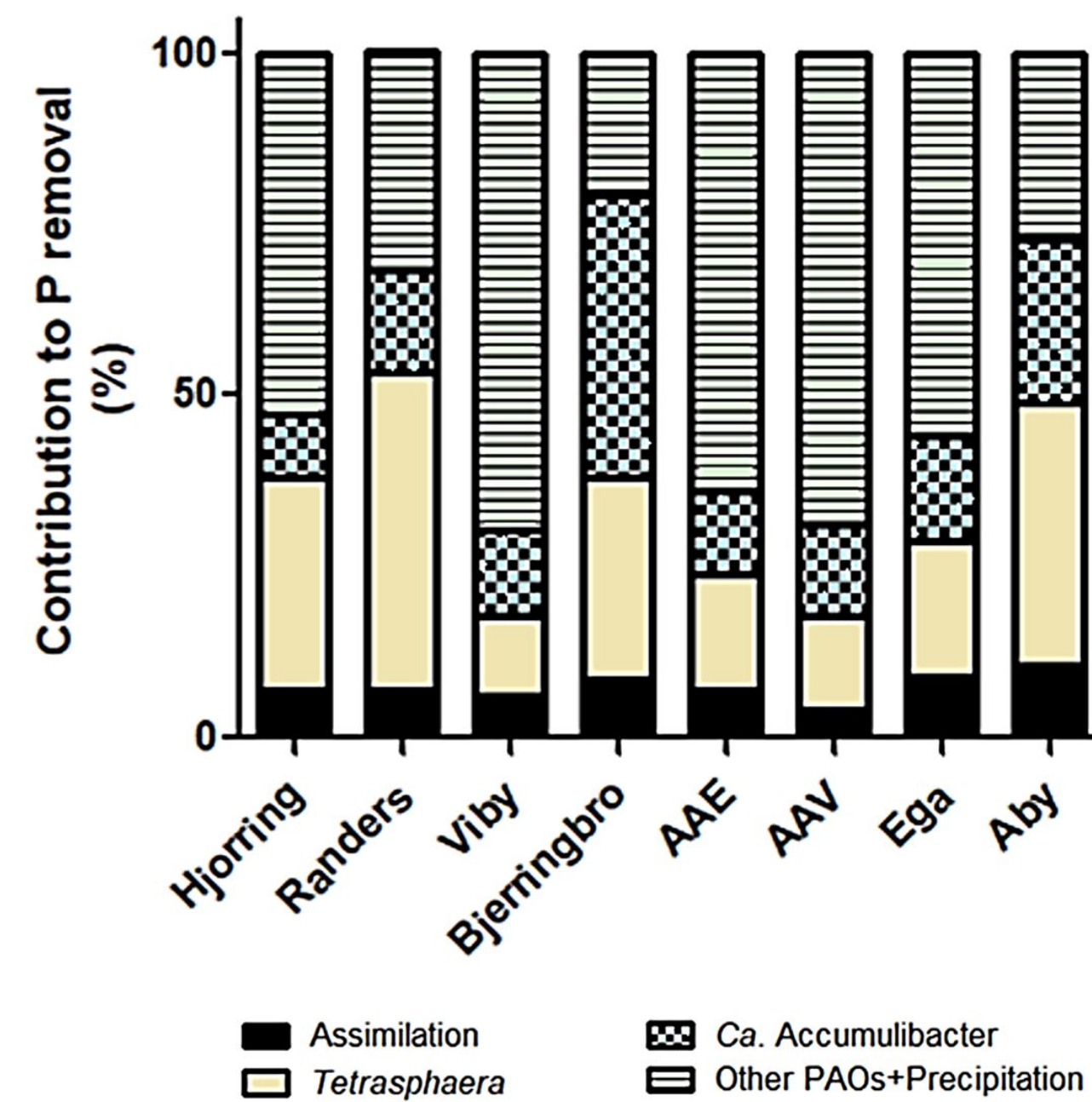


The dynamics of the feast-famine experiment with activated sludge (A) were corroborated by the changes in the storage polymers in *Ca. Accumulibacter* (B and D) and *Tetrasphaera* (C).

## In situ quantification of storage compounds in full-scale EBPR plants



Surprisingly, no PHA or glycogen were found in *Tetrasphaera* and, in most of the plants, its contribution to the total P-removal was higher than that of *Ca. Accumulibacter*. However, they are both key organisms for the EBPR process, storing together up to 70% of the total P present.



fp@bio.aau.dk



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